WE193 Toward an AOP-based tiered testing strategy for thyroid hormone disruption in fish D. Knapen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; E. Stinckens, University of Antwerp; L. Vergauwen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences & SPHERE Dept Biology; J.E. Cavallin, Badger Technical Services, U.S. EPA / National Health and Environmental Effects Research Laboratory; G.T. Ankley, U.S. Environmental Protection Agency / National Health and Environmental Effects Research Laboratory; D.L. Villeneuve, U.S. Environmental Protection Agency / Mid-Continent Ecology Division. Assessment of thyroid hormone disrupting chemicals (THDCs) is considered a major gap in current approaches for the testing of endocrine disrupting chemicals. The thyroid hormone system is involved in several important physiological and developmental processes, and many chemicals found in our environment may interfere with the thyroid system. The need to advance screening and testing strategies for THDCs includes the development of *in silico* and *in vitro* methods that anchor chemicals acting through a thyroid mechanism to adverse responses.The Adverse Outcome Pathway (AOP) framework may therefore be particularly helpful for the identification of relevant assays and endpoints. Here, we demonstrate the use of *in chemico* assays targeting specific key events of an established AOP network to predict higher biological endpoints in fish early-life stages. Specifically, an AOP network linking thyroid hormone disruption to impaired swim bladder inflation was used to select assays measuring thyroperoxidase and deiodinase inhibition, key enzymes in the thyroid hormone metabolism. A set of 51 compounds was screened using these assays, and data were used to predict acute and chronic effects on swim bladder inflation. Predictions were validated using FET and FELS *in vivo* experiments in zebrafish and fathead minnow, and *in chemico* to *in vivo* extrapolation threshold values were established. A tiered testing strategy for the identification of THDCs was proposed based on these data. Our thyroid hormone disruption AOP network is part of the OECD AOP development programme workplan (project 1.35), and the associated assays align with the thyroid-related assays that are listed in the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals (revised 2018) as assays forwhich no formal guidance has been written at present. In addition, as part of their endocrine disruptor screening program, the US EPA included this work while assembling a conceptual thyroid hormone disruption AOP network spanning different taxonomic groups (fish, amphibians, mammals) to assist high throughput assay development. The ongoing JRC EURL ECVAM validation effort of *in vitro* assays for THDCscreening is making use of this project's data to ensure synergies and overlap. Finally, our AOP network will be used as background for the H2020 project ERGO, "Breaking down the wall between human health and environmental testing of endocrine disrupters".